

# This Week's Citation Classic™

**Koelle G B & Friedenwald J S.** A histochemical method for localizing cholinesterase activity. *Proc. Soc. Exp. Biol. Med.* **70**:617-22, 1949. [Wilmer Ophthalmological Institute, Johns Hopkins University and Hospital, Baltimore, MD]

A histochemical method is presented for localizing cholinesterase activity by incubating tissue sections in a medium containing acetylthiocholine, copper glycinate, and copper thiocholine. Results obtained with several tissues containing specific cholinesterase are described and illustrated [The *SC*<sup>®</sup> indicates that this paper has been cited in over 840 publications since 1955.]

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"Following my discharge from the Army in 1946, I joined Jonas S. Friedenwald as half-time Chalfant Fellow in Ophthalmology (for the specific purpose of elucidating the role of adrenochrome in secretion of the aqueous humor), while attending medical school at Johns Hopkins University. The cited publication was a natural outcome of (his collaboration. During the war, I had worked on the anti-cholinesterase nerve gases; Friedenwald, while primarily an ophthalmologist, was also one of the pioneers in enzymatic histochemistry in addition to his many other accomplishments (he was in fact the only genius I have ever known). At a point when the adrenochrome work appeared to have reached an impasse, I suggested to him that we might develop a histochemical method for the localization of cholinesterases. The copper-thiocholine procedure was the result. It has been cited recently (by an old friend) as 'the first long step towards bringing neurohistology into the twentieth century.'<sup>1</sup> The year following its publication, at the instigation

of Julius H. Comroe, I received the Abel prize for a modification which permitted the separate localizations of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).<sup>2</sup>

"The method and its several modifications (the most important of which is that of Karnovsky and Roots<sup>3</sup>) have been used extensively in our laboratory and in many others.<sup>4,5</sup> However, for a number of reasons it is not satisfactory for electron microscopy. For this purpose we developed the bis-(thioacetoxy) aurate method, which affords an extremely high level of resolution but is less specific; accordingly, it requires the use of rather elaborate controls for the accurate localizations of AChE and BuChE.<sup>6</sup> Comparison of results obtained by the two methods, by light and electron microscopy, in the normal and denervated superior cervical ganglion of the cat<sup>7,8</sup> led to the conclusion that a neurotrophic factor is essential for the maintenance of AChE and BuChE at postsynaptic sites in noncholinergic neurons.<sup>9</sup> We have recently demonstrated the presence of such a factor in extracts of the central nervous system,<sup>10</sup> and are now involved in its characterization and determination of its mechanism of action.

"Prior to the publication of our original findings, our colleague at the Wilmer Institute, Stephen Kuffler, asked Friedenwald and me if he might show one of our slides at a symposium he was attending in Spain. We readily agreed. Afterward, Steve told us that the participants were impressed by the picture but baffled by the unknown names of its producers. He enlightened them by identifying us as a medical student and an ophthalmologist. Another ophthalmologist, Bernie Becker, predicted, 'You can work on this for the next 20 years!' After nearly twice that long, I am still at it."

1. **Garrett J R.** Adventures with autonomic nerves. *Proc. Roy. Microscopical Soc.* **17**:242-53, 1982.
2. **Koelle C B.** The histochemical differentiation of types of cholinesterases and their localizations in tissues of the cat. *J. Pharmacol. Exp. Ther.* **100**:158-79, 1950. (Cited 220 times since 1955.)
3. **Karnovsky M J & Roots L. A** "direct-coloring" thiocholine method for cholinesterases. *J. Histochem. Cytochem.* **12**:219-21, 1964. (Cited 1,210 times.)
4. **Koelle G B.** Cytological distributions and physiological functions of cholinesterases. (Koelle G B, ed.) *Cholinesterases and anticholinesterase agents.* Berlin: Springer-Verlag, 1963. p. 187-298.
5. **Silver A.** *The biology of cholinesterases.* New York: American Elsevier Publishing Co., 1974. 596 p.
6. **Koelle G B, Davis R, Smyri E G & Fine A V.** Refinement of the bis-(thioacetoxy) aurate (I) method for the electron microscopic localization of acetylcholinesterase and nonspecific cholinesterase. *J. Histochem. Cytochem.* **22**:252-9, 1974.
7. **Davis R & Koelle G B.** Electron microscope localization of acetylcholinesterase and butyrylcholinesterase in the superior cervical ganglion of the cat. I. Normal ganglion. *J. Cell Biol.* **78**:785-809, 1978.
8. .... Electron microscope localization of acetylcholinesterase and butyrylcholinesterase in the superior cervical ganglion of the cat. II. Preganglionically denervated ganglion. *J. Cell. Biol.* **88**:581-90, 1981.
9. **Koelle G B.** Unsolved questions on the localization of cholinesterases. (Lee C Y, ed.) *Advances in neuropharmacology.* Taipei: Academia Sinica. 1982. p. 37-43.
10. **Koelle G B & Ruch G A.** Demonstration of a neurotrophic factor for the maintenance of acetylcholinesterase and butyrylcholinesterase in the preganglionically denervated superior cervical ganglion of the cat. *Proc. Nat. Acad. Sci. US—Biol. Sci.* **80**:3106-10, 1983.